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Donovanosis in Papua New Guinea

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Summary

Clinical and epidemiological observations on 87 cases of donovanosis seen at Port Moresby General Hospital are presented, with detailed reports of three cases in which the disease was more severe. The circumstances of infection described were consistent with venereal transmission. Chloramphenicol and gentamicin were effective in curing the disease, while streptomycin was found to be ineffective in a number of cases tested. Complement-fixation tests with *Donovania* antigen revealed the presence of antibodies in all but one of 23 cases tested, and in nine out of fourteen other patients who on clinical grounds were suspected of having donovanosis but were negative by smear test. The complement-fixation test with *Klebsiella* antigen was found to be highly specific for donovanosis, but less sensitive than the test using *Donovania* antigen. The intracellular location of *Donovania* in tissue and the presence of antibodies which are apparently not protective suggest that cell-mediated immunity may be important in defence against *Donovania* infection.

Introduction

The causative organism of donovanosis is *Donovania granulomatis* (the Donovan body), which cannot be cultured on ordinary laboratory media but has been successfully cultivated on a few occasions in the yolk sac of embryonated eggs (Anderson, 1943; Dunham and Rake, 1948; Dienst, Greenblatt, and Chen, 1948; Goldberg, Weaver and Packer, 1953), and in an artificial medium containing egg yolk and lactalbumin hydrolysate (Goldberg, 1959). Little is known about the organism except that it has been shown to be antigenically related to *Klebsiella* and to a number of other members of the *Enterobacteriaceae* (Goldberg, 1954; Packer and Goldberg, 1950).

By comparison with other venereal diseases, donovanosis is only mildly contagious. Published reports show a variable but generally low level of transmission of the disease to conjugal partners (Packer and Goldberg, 1950; Rajam and Rangiah, 1954). Racial susceptibility appears to vary, donovanosis being a disease primarily of Negroes in the United States (D'Aunoy and Van Haam, 1937), and occurring more frequently among Hindus than Moslems in India (quoted in Greenblatt, 1947). It is not known, however, whether such differences have a genetic basis or are due to different social habits and standards of personal hygiene associated with low economic status.

Donovanosis has been a clinical problem in Papua New Guinea for over 70 years, and currently 150 to 200 cases are seen annually in Port Moresby. In this communication, a summary of clinical and epidemiological observations on 87 cases are presented, together with the results of serological studies on some of them.

Material and methods

Subjects

Patients with ulcerative lesions of the genitalia presenting to the General Hospital or to urban clinics in the town area of Port Moresby were encouraged to accept admision to hospital. Of 160 such patients admitted over a 14-month period, 87 were shown to have donovanosis and are the subjects of this study. The case of disseminated donovanosis described here was seen outside this 14-month period.

Bacteriological diagnosis

A small piece of granulation tissue was removed from the surface of the lesion using nasal punch biopsy forceps, crushed between two slides, and stained with Wright's stain. A positive diagnosis was made on finding characteristic bipolar staining capsulated bacilli (Donovan

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bodies) within the cytoplasm of macrophages (Fig. 1). Smears made by scraping the surface of the lesion with a scalpel blade are unsatisfactory because of the large numbers of contaminating bacteria.

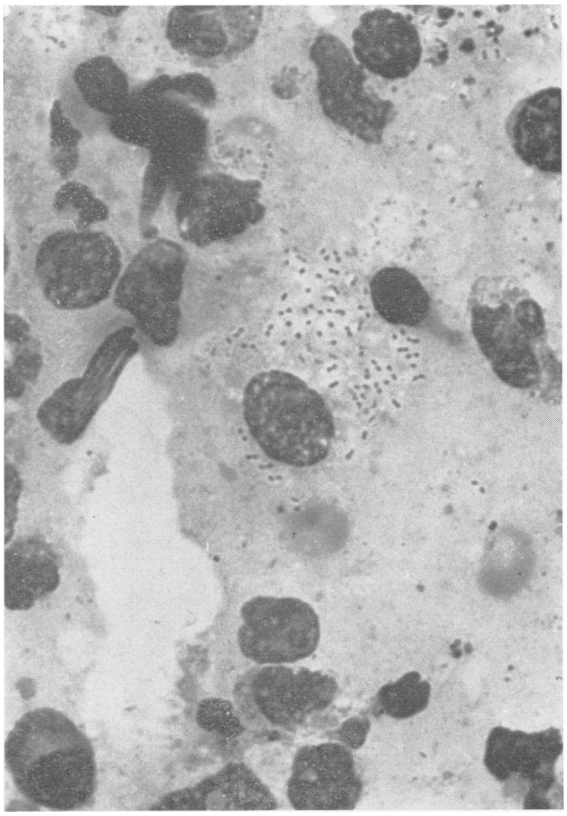

FIG. 1 *Donovan bodies seen in a smear of a small piece of granulation tissue from a donovanosis lesion. Stained with Wright's stain*

Serology

Complement-fixation tests were carried out using a Microtitre apparatus (Cooke Engineering Co., California, U.S.A.) with complement and haemolysin obtained from Commonwealth Serum Laboratories, Australia. *Donovania* antigen (Anderson strain), kindly provided by Dr Julius Goldberg, was a washed cell suspension preserved with merthiolate. For the preparation of the *Klebsiella* antigen, an 18-hour growth of the organism on nutrient agar slopes was harvested, washed three times in phosphate buffered saline (PBS), and re-suspended finally in a volume of PBS equivalent to 3 ml. per 5 ml. agar in the slope. The cell suspension was autoclaved and diluted a further ten-fold before use.

Culture attempts

Tissue, or the aspirate from a pseudobubo, was inoculated into the yolk sac of 4 to 6-day-old embryonated eggs and, in some cases, into the lactalbumin hydrolysate-egg yolk medium described by Goldberg (1959). Eggs were obtained from two sources, one of which was known to use antibiotic-free poultry feed. Tissue was prepared for inoculation by teasing it out into small fragments which were incubated overnight on blood agar or in tissue culture medium. Those tissue fragments which were not contaminated with other organisms were used for inoculation after suspension in saline.

Results

Age and sex distribution

Ten of the 87 patients were female. The ages, which ranged from a girl of 10 to a man of 55, were often estimates; they are shown in Table I.

TABLE I *Age and sex of 87 patients*

	Sex	
Age group (yrs)	*Male*	*Female*
10–19	8	2
20–29	42	6
30–39	19	—
40–49	6	1
50+	2	1

Origin

All the patients were indigenous Papua New Guineans. All but one came from outside the Port Moresby urban area, and most had been living in the town for more than one year. The distribution of place of origin of the cases roughly paralleled that of Papuans and New Guineans from outside Port Moresby who were living in the town at the time of the 1966 census. The majority were unschooled labourers; only two had advanced beyond primary school.

Duration of disease

49 patients claimed that their ulcer had been present for less than a month, and 32 that it had been present from 1 to 12 months, and six had noted ulceration for more than one year.

Sexual history

Most male patients admitted to sexual contact, usually with a prostitute. Most of the female patients denied casual sexual contact, and three denied any contact. No attempt was made to trace contacts beyond asking married patients whether the spouse had similar lesions. Two patients were husband and wife. It was difficult to obtain a reliable estimate of the incubation period because of frequent sexual contacts in some cases, denial of contact in others, and the length of time elapsing between the appearance of the lesions and presentation at the hospital. Our impression, however, is that the incubation period varies from weeks to months.

Lesions (Table II)

Red exuberant lesions were most likely to be positive on smear, but other flat or irregular lesions were occasionally positive. Ulceration in the groin was relatively common and, when this was present, Donovan bodies were nearly always demonstrated. Other patients had inguinal swelling without ulceration which was regarded as lymph node enlargement due to secondary infection of the genital ulcer. Inguinal pseudobuboes were seen in two patients.

TABLE II *Distribution of lesions (males)*

Site	*No. of patients with lesions*
Frenulum	29
Prepuce	55
Glans	27
Shaft	29
Groin	23 (swelling in a further 18)
Anus	4

Ulceration was occasionally very extensive, covering an area of up to 50 sq. cm. In one patient (Case 1, see below), complete erosion of the penis had occurred (Fig. 2).

Of the four patients with anal lesions (Fig. 4), none had penile lesions although one showed extensive ulceration of the groin. Three of the four admitted homosexual contact.

In the ten females, seven had involvement of the fourchette and labia, and three the vagina and/or cervix. In one patient, all four areas were involved together with the clitoris, vestibule, and groin.

Three of the 87 patients were admitted originally to the surgical ward with a provisional diagnosis of carcinoma.

Extragenital lesions

One patient had an extragenital lesion in the neck together with groin lesions (Case 2, see below).

Disseminated donovanosis

A number of cases of disseminated donovanosis have been seen in Port Moresby over the past years, although none presented in the 14-month period of this study. A patient seen since that time is described below (Case 3).

Case reports

Case 1 Destruction of the penis was the main presenting feature in this case.

An unmarried hospital labourer aged about 30, who had worked at the Port Moresby General Hospital for over 10 years, had first noticed a penile ulcer 12 months previously, but stated that this had healed after receiving penicillin injections. An ulcer recurred 3 months before admission to hospital, beginning on the middle of the shaft of the penis, and extending both proximally and distally. At the same time an ulcer appeared on the lower abdominal wall above the root of the penis.

The ulceration had destroyed all except 2 to 3 cm. of the base of the penis, which was replaced by bright pink granulation tissue, in the middle of which a small island of epithelium marked the urethra. There was an area of granulation tissue 5 cm. in diameter above the root of the penis (Fig. 2). The inguinal glands were not enlarged.

FIG. 2 *Case* 1. *Erosion of penis by donovanosis lesion*

Donovan bodies were demonstrated in smears made from a punch biopsy. Treatment was started with chloramphenicol 500 mg. four times a day and 6 days later the penile granulations were dry and shrinking and centripetal healing of the abdominal lesion was progressing rapidly. After 11 days treatment, only a small area of granulation remained uncovered by epithelium, and this had healed one week later. There has been no recurrence.

This case demonstrates the capacity for destruction of tissue which donovanosis manifests in a few patients. Such destruction is uncommon, and can be confused with amoebic balanitis. No amoebae were seen in wet preparations from the surface of this lesion.

Case 2 An extragenital lesion together with long-standing inguinal lesions was the main presenting feature in this case.

A 50-year-old Papuan man had been seen on several occasions before being admitted to the present series. He had first noticed an ulcer on the glans penis in 1965. Several months later he became aware of tenderness in

the right groin and here the skin broke down in a long ulcer along the inguinal fold which was overhung by his obese abdominal wall. This ulcer persisted in spite of saline baths and penicillin injections.

He was admitted to hospital in 1967 with an extensive area of bright red granulations in the right groin, weeping serosanguineous exudate. Donovan bodies were demonstrated in tissue smears and streptomycin was given, 4 g daily, to a total dose of 20 g. The patient asked to go home for family reasons and did not complete the course. Although the lesions had regressed, the groin ulcer did not heal completely.

He was seen again in 1970 when he had an area of ulceration over the left side of the neck, extending up over the edge of the mandible. Donovan bodies were present in this lesion and also in ulcers present in both groins. Treatment with streptomycin produced only slow healing, and tetracycline, 2 g daily, was begun. This seemed to clear the lesion of Donovan bodies, but healing was still very slow. He was discharged to continue treatment as an outpatient, and 2 months later the lesions (which had not healed completely) were again active, red and weeping, and the right side of the face was puffy. Donovan bodies were again present in the lesions. On this occasion streptomycin 24 g, metronidazole 28 g, ampicillin 20 g, and a course of "Stibophen" all failed to produce significant improvement and Donovan bodies persisted in repeated smears. The patient elected to go home. He was recalled to the present study when a supply of gentamicin became available 3 months later, and was admitted to hospital. Under general anaesthesia, the left facial lesion was curetted of all its heaped-up granulation tissue. Gentamicin, 1 mg/kg twice daily, was given for 11 days. On this occasion, healing was more rapid and the neck lesion was completely healed 1 month later. The groin ulcers persisted for a further 18 months, finally resolving after a course of chloramphenical, 2 g daily, for about 6 weeks.

Lack of evidence of any bone involvement in this patient suggests autoinoculation as the most likely route of infection for the neck lesion. This case suggests the importance of the local environment for the persistence of donovanosis lesions. The patient was short and obese (96 kg) and his groins and neck were deeply folded and moist.

Case 3 The occurrence of bone lesions, one of which was deep to normal skin, strongly suggested spread in the blood stream in this case.

A 15-year-old boy presented with suppurating ulcers affecting the perianal area and both groins. He stated these had first appeared 2 weeks previously. He also had an exuberant mass of red granulation tissue 3 cm in diameter on the medial side of the left arm above the elbow. He denied any sexual contact. Tissue from all the ulcers contained Donovan bodies; 2 days after admission he complained of pain over the upper anterior surface of the left tibia where the skin covered a fluctuant area. Radiography showed an erosion of the underlying tibia (Fig. 3). A similar erosion was present in the humerus under the ulcer there. These lesions were both curetted under general anaesthesia and treatment was started with chloramphenicol 2 g daily. Improvement was very slow but continuous over the following 2 weeks, but

FIG. 3 *Case* 3. X *ray of eroded left tibia*

the patient left hospital and 3 months later returned with two huge ulcers over both tibiae and extensive lesions in both groins, around the anus (Fig. 4) and over the humerus. Surgical removal of the heaped-up granulations was again carried out, and chloramphenicol started as before. Again, however, the patient discharged himself before healing was well advanced.

Treatment

Twelve of the 87 patients absconded before the effects of treatment could be assessed. The treatment previously recommended in Papua New Guinea for donovanosis was streptomycin 4 g. daily for 5 days (Maddocks, 1967). The first fourteen patients received streptomycin for 1 to 5 weeks (average dose 36 g.). One patient showed complete healing and in only four others was there thought to be progressive clinical improvement. Six remained smear positive after receiving streptomycin.

The standard treatment was changed to chloramphenicol 500 mg. four times a day. Fifty cases were treated and 43 showed complete or near-complete clinical and bacteriological healing with an average dose of 33·6 g. chloramphenicol.

Although there was concern about the risk of agranulocytosis during prolonged treatment with

FIG. 4 *Case 3. Extensive ulceration of tibiae and in both groins and around anus.*

chloramphenicol (up to 10 weeks in some cases), financial considerations and the non-effectiveness of streptomycin made it a drug of choice. White cell counts were followed in patients receiving large doses, and were not found to be affected.

A gift of gentamicin (Garamycin) was received from Schering Co. with the object of determining the efficacy of this drug in donovanosis. Preliminary use of gentamicin (1 mg./kg. three times a day) in patients not responding to streptomycin showed it to be effective in most cases.

In a more definitive comparison with chloramphenicol, patients were alloted alternately to chloramphenicol and gentamicin treatment groups, and healing was assessed clinically and bacteriologically twice weekly. Chloramphenicol produced slightly faster clinical healing and disappearance of organisms (Table III).

TABLE III *Comparison of efficacy of chloramphenicol and gentamicin in donovanosis*

Drug	*Chloramphenicol*	*Gentamicin*
Number of cases	13	9*
Mean time to become smear negative (days)	7	12
Mean dose (g) to smear negative	12	1·4
Mean time to be healed or discharged (days)	17	22
Mean dose to be healed or discharged(g)	29	2·4

*A further three patients on gentamicin defaulted

Culture attempts

Because of the desirability of having locally prepared antigen, culture of *Donovania granulomatis* was attempted. Tissue from seven patients, and aspirate from the pseudobuboes of a further two, all taken before starting treatment, were inoculated into eggs and, in some cases, into lactalbumin hydrolysate-egg yolk medium. The yolk of inoculated eggs was further passaged in eggs up to four times at weekly intervals. On no occasion was culture of *Donovania* achieved, as judged by the microscopical examination of stained smears.

Serology

As we were unable to establish a culture of a local strain of *Donovania*, a preparation of *Donovania* antigen (Anderson strain) provided by Dr. J. Goldberg was used for serological studies. Complement-fixation tests with this antigen were carried out on sera from 28 patients with bacteriologically diagnosed donovanosis (Group I). Tests were also done on fourteen cases that clinically resembled donovanosis but had been excluded from the present study because of a negative smear on microscopy, and also on five controls of whom three had no venereal disease, one had gonorrhoea, and one venereal warts. The results are shown in Table IV. All but one of the cases in Group I had an antibody titre of 1: 5 or greater, as did nine of the fourteen cases in Group II. None of the control sera had detectable antibody.

TABLE IV *Complement-fixation test using* Donovania *antigen*

Group	*No. of cases*	*No. with titre* > 1:5
I Bacteriologically proven donovanosis	28	27 (range 1:10 to 1:2560)
II Clinically resembling donovanosis, smear negative	14	9 (range 1:5 to 1:320)
III Controls	5	0

As there was insufficient *Donovania* antigen for routine testing, the use of *Klebsiella* as antigen was investigated. The organism used was a strain of *K. pneumoniae* isolated from a urinary tract infection. The results are shown in Table V. Complement-fixing antibodies were found in sixteen out of 23 cases in Group I and in three out of twelve cases in Group II. None of 31 control sera showed a detectable level of antibody. These latter sera were obtained

TABLE V *Complement-fixation test using* Klebsiella *antigen*

Group	*No. of cases*	*No. with titre* > 1:5
I Bacteriologically proven donovanosis	23	16 (range 1:5 to 1:640)
II Clinically resembling donovanosis, smear negative	12	3 (range 1:40 to 1:80)
III Controls	31	0

from healthy volunteers and from patients with gonorrhoea, syphilis, or medical conditions not related to venereal disease, including patients from whom *Klebsiella* had been isolated from sputum, urine, or pus.

Titres were uniformly lower to the *Klebsiella* than to the *Donovania* antigen. Sera in Groups I and II that were positive to *Donovania* and negative to *Klebsiella* tended to be those with lower titres to *Donovania*. Those in Group II that were negative to *Donovania* were also negative to *Klebsiella*. All sera with a titre of 1: 80 or more to *Donovania* showed complement-fixing antibodies to *Klebsiella*. With the *Klebsiella* antigen, the complement-fixation test was thus specific for donovanosis but less sensitive than the test using *Donovania* antigen.

Discussion

The circumstances of infection in cases of donovanosis in Port Moresby are consistent with venereal transmission of the disease. The type of patient presenting most commonly was a young, single, healthy man, who gave a clear history of recent sexual contact with a prostitute. Two of the cases studied were husband and wife. Goldberg (1959) claimed to demonstrate a faecal habitat for the *Donovania* bacillus and suggested transmission by pederasty to explain the low infectivity of the disease. Histories of heterosexual and homosexual contact in this series were not reliable and did not clarify the problem of non-sexual transmission of donovanosis.

Most of the patients were unschooled labourers who had migrated to Port Moresby from other parts of Papua New Guinea and were living in settlements with limited hygiene facilities. Donovanosis is extremely rare amongst the European population of Port Moresby, which comprises 20 per cent. of the total population of the town. The restriction of the disease to the indigenous population need not necessarily imply any racial difference in susceptibility. European men usually seek sexual contacts with women from a better social class and with a higher level of education and hygiene than the relatively poor men who develop donovanosis.

Chloramphenicol was the most effective drug used; Gentamicin was also effective but streptomycin was found to be largely ineffective, suggesting that a streptomycin-resistant strain of *Donovania granulomatis* may have developed.

Surgical removal of granulations probably assists chemotherapy to overcome infection that is slow to respond. The neck lesion in Case 2 is an example, in which the local environment of the lesion probably hindered healing of the mass of granulation tissue. Surgical treatment by itself, however, is generally not effective unless it is radical. In Case 3 also, surgery seemed likely to hasten the response to chemotherapy, but the patient was unwilling to submit to prolonged inpatient supervision, perhaps because the rather slow response caused him to lose faith in the efficacy of the regime.

The strain(s) of *Donovania granulomatis* causing donovanosis in Papua New Guinea appears to be closely related to the North American Anderson strain, since all but one of the bacteriologically proven cases of donovanosis tested possessed complement-fixing antibodies reacting with the North American antigen preparation, often in high titre. The presence of antibody to *Donovania* in nine out of fourteen clinically positive but bacteriologically negative patients indicates a diagnosis of donovanosis in these patients also, since control sera lacked such antibodies. In the present study, only a small number of control sera were tested, owing to shortage of antigen and our wish to test as many patients as possible; however, a number of other workers have reported the absence of complement-fixing antibodies to *Donovania* in large control series (Goldberg and others, 1953; Dunham and Rake, 1948). The complement-fixation test with *Klebsiella* as antigen showed a high specificity for donovanosis but was not sufficiently sensitive to be useful as a diagnostic test. Possibly the use of a different strain of *Klebsiella* would render the test more sensitive.

The majority of cases of donovanosis can be diagnosed by smear and a serological test is not required. The complement-fixation test with *Donovania* antigen would, however, assist in the diagnosis of doubtful cases where the smear is negative, particularly in cases where the differential diagnosis is carcinoma. The test could also be of value in epidemiological studies. It is not clear why we were not successful in our attempts to culture *Donovania* using tissue or pus rich in the organism. A possible factor is the composition of the feed given to the hens producing the eggs; Goldberg and others (1953) stressed the importance of a 'natural' (on range) diet for the hens for the production of eggs that would support the growth of *Donovania*, and it is possible that the processed feed used in the present study lacked an essential growth factor.

Donovanosis is a chronic disease, showing little or no tendency towards spontaneous healing. The presence in patients of high levels of circulating antibodies which are not protective is similar to the situation found in a number of other diseases in which the causative organism is intracellular, *e.g.*

lepromatous leprosy, tuberculosis, and chronic mucocutaneous candidiasis. By analogy with these diseases, it seems likely that protection against donovanosis is afforded by the cellular immune system.

Donovanosis is apparently not highly infectious. We did not trace many contacts in the present study, but several workers have reported only a low level of transmission of the disease to conjugal partners (Packer and Goldberg, 1950; Goldberg, 1964). A high dose of the organism deposited on an already existing traumatic lesion may be required for infection to occur. A further possibility is that people who develop clinical donovanosis, and particularly the disseminated form, are in some way deficient in their cellular immune response to the organism, while the majority of contacts have an adequate cellular response and have only subclinical infection. Assays of cellular immunity using *Donovania* antigen should provide interesting information on the pathogenesis and epidemiology of this disease. Failure to culture the organism has so far prevented us from pursuing this aspect of the study.

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